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Molecular characterization of estrogen receptors with focus on breast cancer

AKADEMISK AVHANDLING

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ABSTRACT

Estrogen signaling is mediated by estrogen receptors (ERs), ER α and ER β . Aberrant estrogen signaling is involved in breast cancer development. ER α is one of the key biomarkers for diagnosis and treatment of breast cancer. Unlike ER α , ER β is still not introduced as a marker for diagnosis and established as a target of therapy. Numerous studies suggest antiproliferative effects of ER β , however its role remains to be fully explored. Albeit important, ER α is not a perfect marker, and some aspects of ER α function are still unclear. This thesis aims to characterize distinct molecular facets of ER action relevant for breast cancer and provide valuable information for ER-based diagnosis and treatment design.

In **PAPER I**, we analyzed the functionality of two common single nucleotide polymorphisms in the 3' untranslated regions of ER β , rs4986938 and rs928554, which have been extensively investigated for association with various diseases. A significant difference in allelic expression was observed for rs4986938 in breast tumor samples from heterozygous individuals. However, no difference in mRNA stability or translatability between the alleles was observed.

In **PAPER II**, we provided a more comprehensive understanding of ER β function independent of ER α . A global gene expression analysis in a HEK293/ER β cell model identified a set of ER β -regulated genes. Gene Ontology (GO) analysis showed that they are involved in cell-cell signaling, morphogenesis and cell proliferation. Moreover, ER β expression resulted in a significant decrease in cell proliferation.

In **PAPER III**, using the human breast cancer MCF-7/ER β cell model, we demonstrated, for the first time, the binding of ER α/β heterodimers to various DNA-binding regions in intact chromatin.

In **PAPER IV**, we investigated a potential cross-talk between estrogen signaling and DNA methylation by identifying their common target genes in MCF-7 cells. Gene expression profiling identified around 150 genes regulated by both 17 β -estradiol (E2) and a hypomethylating agent 5-aza-2'-deoxycytidine. Based on GO analysis, CpG island prediction analysis and previously reported ER binding regions, we selected six genes for further analysis. We identified BTG3 and FHL2 as direct target genes of both pathways. However, our data did not support a direct molecular interplay of mediators of estrogen and epigenetic signaling at promoters of regulated genes.

In **PAPER V**, we further explored the interactions between estrogen signaling and DNA methylation, with focus on DNA methyltransferases (DNMT1, DNMT3a and DNMT3b). E2, via ER α , up-regulated DNMT1 and down-regulated DNMT3a and DNMT3b mRNA expression. Furthermore, DNMT3b interacted with ER α . siRNA-mediated DNMT3b depletion increased the expression of two genes, CDKN1A and FHL2. We proposed that the molecular mechanism underlying regulation of FHL2 and CDKN1A gene expression involves interplay of DNMT3b and ER α .

In conclusion, the studies presented in this thesis contribute to the knowledge of ER β function, and give additional insight into the cross-talk mechanisms underlying ER α signaling with ER β and with DNA methylation pathways.